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TITLE: DNA Copy Number Signature to Predict Recurrence in Early-Stage Ovarian Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT : The first year of this award included the following tasks

Major Task 1: Obtain DNA samples from consortium specimens

- Subtask 1 Pathological review of 592 early-stage high-grade ovarian cancer specimens and select 330 samples for CNV analysis. Months:1 - 2
- Subtask 2 Prepare sections (10 µm) for microdissection to ensure>80% tumor. Months 3 - 8
- Subtask 3 DNA preparation from microdissected specimens. Months 3 - 8

Major Task 2: To determine the copy number gain and loss for early stage high grade ovarian cancers through IlluminaHumanOmniExpress-FFPE BeadChip system

- Subtask 1 DNA sample quality assessment and processing (FFPE restoration) to prepare chip compatible samples. Months: 9 - 18
- Subtask 2 Genomic abnormality analysis by IlluminaHumanOmniExpress-FFPE BeadChip system. Months 9 - 18

Milestone(s) Achieved: *Obtained DNA samples from 330 recurrent and non-recurrent early-stage ovarian cancers for the proposed copy number study.* 96 of these samples have undergone QC quality analysis and 48 have been hybridized to detect CNV. New batches of 48 samples each are being processed for quality analysis and subsequent CNV.

15. SUBJECT TERMS
Early Stage Ovarian Cancer, genomic predictive signature, recurrence

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Introduction

The survival of women with high-grade epithelial ovarian cancer is directly related to the spread of the tumor. Women with disease limited to the pelvis do well with many being cured, while those patients whose tumor has spread outside of the pelvis suffer recurrences and the majority will die from the disease. Nevertheless, the standard of care for patients with high-grade ovarian cancer is surgery followed by 6 cycles of chemotherapy (carboplatin/taxol) regardless of the spread of the tumor. Although some early stage patients are benefiting from this strategy, approximately 50-60% of patients with high-grade early stage cancer will not develop recurrent disease even in the absence of chemotherapy. These patients thereby suffer unnecessary short and long-term toxicities of chemotherapy with no benefit. Thus, the development of accurate biomarkers predictive of tumor recurrence becomes essential to identify women with early-stage disease who will benefit from chemotherapy while sparing the rest the unnecessary treatment with quality-of-life and cost-effectiveness ramifications. This approach parallels efforts in breast cancer where tests like "oncotype dx" provide valuable information on disease recurrence to women with early stage breast cancer. To the best of our knowledge, there is no available large-scale molecular characterization of early-stage ovarian tumors. Here, we propose to develop genomic signatures correlated with clinical outcomes and in particular tumor recurrence for early stage ovarian cancer. The status of DNA copy number variation in recurrent and non-recurrent early stage high grade ovarian cancer will be investigated using a large, fully annotated, consortium cohort of 1628 samples from clinical trials. Integrated analysis will be performed by combining the gene expression profiles obtained from a recently terminated ongoing DOD project (W81XWH-12-1-0521) using the same 1628 samples with the gene copy results from this proposal. Through the integrated analysis of deleted, amplified and aberrantly expressed genes in early stage ovarian cancer, we expect to develop predictive biomarkers for future prospective stratification of women with early stage ovarian cancer to adjuvant carboplatin/taxol chemotherapy versus careful follow-up. This study will also contribute the identification of therapeutic biomarkers and stratification of early stage ovarian cancer patients most likely to benefit from targeted interventions.

KEYWORDS: Early Stage Ovarian Cancer, genomic predictive signature, recurrence, DNA copy number variation

Research Accomplishments

The tasks for the first project period included: 1) Obtain DNA FFPE specimens collected through the consortium of early stage high grade ovarian cancer, 2) Analyze about 50% of all required samples through IlluminaHumanOmniExpress-FFPE BeadChip system.

Through a previous DOD award (W81XWH-12-1-0521) we have: 1) Established an international consortium through which we have collected 1628 FFPE samples of serous ovarian cancer, 2) identified 592 early-stage high-grade ovarian cancers with 5-year follow-up, clinical annotation and accurate pathological review (228 recurrent and 364 non-recurrent), 3) established a specimen repository and clinical data inventory at MGH, 4) Sequenced RNA from these tumor samples.

Preliminary RNAseq analysis has indicated the need of analyzing at least 300 samples with a 2:1 ratio (2 control (non recurrent) cases for each recurrent case) to obtain a statistically significant genomic signature. In addition we have estimated a potential 30% loss of samples due to poor DNA extraction. We have thus decided to extract DNA from 220 non recurrent and 110 recurrent early-stage high-grade ovarian cancers with 5-year follow-up. To do this, we have first requested IRB approval for using the cancer FFPE samples collected by the consortium to identify molecular features that distinguish recurrent and non-recurrent tumors through analysis of DNA copy number variation. The IRB protocol was submitted to HRPO for further approval. Once approved, we proceeded with selection of the tissues for our analysis.

Development of a standardized protocol for FFPE DNA extraction: To select the tissues that would be used for analysis of DNA copy number variation, we have analyzed the H&E staining of the 592 early-stage high-grade ovarian cancers identified during the previous award project to select for samples with equal amount of tissue and similar stroma versus epithelium ratio. Slides from selected samples were then stained with cresyl violet, to ensure similarity with the tissue observed through H&E stain and to perform guided macro-dissection and ensure at least 80% tumor cell content within the samples subjected to nucleic acid extraction. Cresyl violet forms non-covalent, easily-reversible binding to nucleic acids and allows distinguishing tumor tissue from stroma. The staining provided by Cresyl violet is comparable to traditional dyes such as hematoxylin but, unlike hematoxylin, it does not chemically modify DNA or RNA and interfere with downstream profiling study (Figure 1). DNA extraction from FFPE sections was then carried out through the use of QIAGEN QIAamp® DNA FFPE Tissue Kit (56404). De-paraffinized, macrodissected FFPE tissues were briefly digested with Proteinase K in Buffer PKD (QIAGEN) to release the RNA into solution while the after-digestion pellet contained primarily DNA that was processed by the QIAGEN DNA FFPE kit.

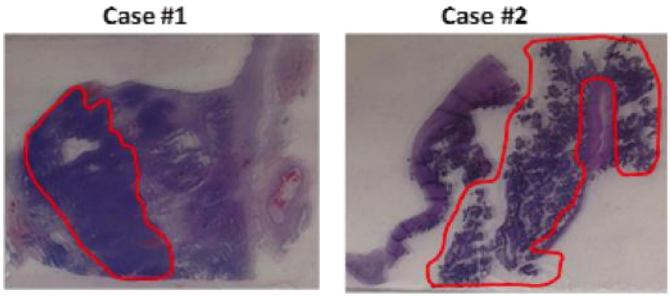

Figure 1. Cresyl violet guided macrodissection to enrich tumor component (circled by red line). In brief, deparaffinized and rehydrated FFPE tumor sections were briefly dipped into 0.5% (v/v, dissolved in 50% EtOH) cresyl violet for 30 seconds. Excessive dye was washed sequentially by 70% and 90% EtOH. Sections were then dehydrated in 100% EtOH and air-dried before macro-dissection.

Procedures for "Batch effect" control: Considering the relative large size of the proposed study, we noticed that the batch effect might have significant impact on data analysis for our signature development. In consultation with Dr. Victoria Wang, biostatistician from the Dana Farber Cancer Institute, we have established a two-tier of strategy to reduce the batch effect. 1) From a bioinformatics prospective, standard surrogate variable analysis / principle component analysis (sva/pca) is used to estimate artifacts introduced by factors irrelevant to biology such as sample source, sample age and technical variations. 2) We also set up SOP to minimize the technical variations during the wet-lab procedures. The latter includes: 1) performance of all extractions by only one dedicated post-doctoral fellow, and 2) tight quality control (e.g. repetitive assaying of the same sample). To perform the bioinformatics analysis of potential

batch effects we have generated a fully annotated sample datasheet that records the following parameters for each sample: tumor block age, cutting-to-extraction time, tumor volume used for extraction (estimation based on number of 10µm slides), tumor purity (70 to >90% purity), DNA and RNA yield, type of stromal pattern, stromal versus tumor TILs infiltration pattern.

Selection of Next generation sequencing core facility for DNA-CNV: 10 tumor samples (5 recurrent and 5 non recurrent) were analyzed at 2 different core facilities: 1) The Partners Translational Core in Cambridge MA, 2) RPCI Genomics Shared Resources at Roswell Park Cancer Institute. Results for the two core facilities were similar. We selected the latter one for our analysis, mainly due to better managerial efficacy.

We have then submitted an initial batch of 73 samples to this core facility and were assayed for quality control (Table 1). Of these, 6 were discarded due to poor quality, 48 were selected for the first round of hybridization and the remaining 19 will be used in the next round of hybridization. Please note that the technology is set in a way that 48 samples are analyzed at once. The list of samples and related results are attached

Table 1: Quality control of DNA samples and selection for CNV analysis

	User			GSR				
Customer Sample ID	Amount (ng)	Conc (ng/uL)	Control?	Conc (ng/uL)	Vol (uL)	Yield (ng)	1ng/uL Dil Vol (uL)	QC Score
A1	1005.3	99.5	No	40.33	9.1	367	39.3	1.98
A2	1011.4	101.1	No	78.24	9	704	77.2	2.17
A3	503.9	48.9	No	43.955	9.3	409	43.0	2.28
A4	1001.0	57.2	No	127.29	16.5	2100	126.3	1.27
A5	1003.1	57.7	No	49.91	16.4	819	48.9	4.04
A6	1005.3	100.5	No	134.315	9	1209	133.3	3.18
A7	1010.2	101.0	No	56.195	9	506	55.2	2.85
A8	1012.2	101.2	No	82.43	9	742	81.4	2.20
A9	1000.1	100.0	No	64.995	9	585	64.0	2.85
A10	501.6	50.2	No	35.89	9	323	34.9	3.97
A11	501.5	50.2	No	34.89	9	314	33.9	2.94
A12	504.4	50.4	No	38.925	9	350	37.9	2.44
B1	1000.5	72.5	No	111.965	12.8	1433	111.0	3.47
B2	1004.4	77.9	No	87.1	11.9	1036	86.1	3.61
B3	1012.9	101.3	No	92.33	9	831	91.3	4.16
B4	1006.5	100.7	No	90.185	9	812	89.2	1.68
B5	1004.0	90.5	No	194.49	10.1	1964	193.5	3.23
B6	1003.0	96.4	No	139.405	9.4	1310	138.4	2.94
B7	1023.1	102.3	No	87.09	9	784	86.1	2.11
B8	1069.6	107.0	No	31	9	279	30.0	3.78
B9	1005.9	100.6	No	71.66	9	645	70.7	3.74
B10	504.9	50.5	No	54.74	9	493	53.7	1.00

B11	506.9	50.7	No	33.7	9	303	32.7	4.15
B12	501.6	50.2	No	74.67	9	672	73.7	2.90
C1	1003.8	100.4	No	203.87	9	1835	202.9	1.92
C2	1007.6	100.8	No	83.08	9	748	82.1	2.92
C3	1003.4	97.4	No	78.36	9.3	729	77.4	4.07
C4	1007.3	75.7	No	76.205	12.3	937	75.2	1.63
C5	1000.1	100.0	No	72.355	9	651	71.4	3.53
C6	500.2	46.3	No	53.985	9.8	529	53.0	2.34
C7	504.2	50.4	No	57.735	9	520	56.7	3.48
C8	503.4	50.3	No	70.665	9	636	69.7	2.43
C9	2090.7	209.1	No	80.53	9	725	79.5	1.96
C10	2013.3	201.3	No	32.12	9	289	31.1	2.80
C11	2091.4	209.1	No	63.055	9	567	62.1	2.30
C12	2065.0	206.5	No	67.215	9	605	66.2	2.39
D1	2055.8	205.6	No	56.87	9	512	55.9	3.50
D2	2019.6	202.0	No	41.675	9	375	40.7	3.79
D3	2171.5	217.2	No	66.475	9	598	65.5	3.93
D4	2142.5	214.3	No	66.43	9	598	65.4	2.47
D5	2018.2	201.8	No	85.25	9	767	84.3	3.20
D6	2081.4	208.1	No	59.3	9	534	58.3	4.01
D7	2073.0	207.3	No	56.64	9	510	55.6	1.67
D8	2111.2	211.1	No	82.215	9	740	81.2	3.20
D9	2194.0	219.4	No	49.895	9	449	48.9	0.48
D10	2043.5	204.3	No	57.72	9	519	56.7	1.27
D11	2270.7	227.1	No	44.475	9	400	43.5	1.48
D12	2224.5	222.5	No	64.015	9	576	63.0	2.28
E1	2114.2	211.4	No	84.625	9	762	83.6	2.77
E2	2129.4	212.9	No	98.25	9	884	97.3	2.91
E3	2079.2	207.9	No	63.935	9	575	62.9	1.97
E4	2011.2	201.1	No	59.535	9	536	58.5	2.76
E5	1740.0	174.0	No	79.735	9	718	78.7	2.44
E6	2107.2	210.7	No	40.83	9	367	39.8	1.11
E7	2142.8	214.3	No	102.24	9	920	101.2	2.19
E8	2105.4	210.5	No	67.485	9	607	66.5	3.66
E9	2002.2	200.2	No	49.01	9	441	48.0	3.50
E10	2121.9	212.2	No	91.215	9	821	90.2	4.38
E11	2063.0	206.3	No	48.05	9	432	47.1	2.31
E12	2033.4	203.3	No	30.8	9	277	29.8	5.10
F1	2049.2	204.9	No	11.4	9	103	10.4	3.50
F2	2309.7	231.0	No	111.85	9	1007	110.9	2.47
F3	2065.4	206.5	No	37.6	9	338	36.6	3.65

F4	2068.7	206.9	No	16.3	9	147	15.3	2.85
F5	2053.7	205.4	No	120.065	9	1081	119.1	1.83
F6	2042.5	204.2	No	13.6	9	122	12.6	7.82
F7	2027.9	202.8	No	137.485	9	1237	136.5	2.11
F8	2033.7	203.4	No	43.93	9	395	42.9	2.74
F9	2012.1	201.2	No	7.2	9	65	6.2	5.26
F10	2004.9	200.5	No	15.1	9	136	14.1	3.15
F11	2025.3	202.5	No	87.925	9	791	86.9	1.80
F12	2147.0	214.7	No	31	9	279	30.0	4.53
G1	3120.0	208.0	Yes	185.585	14	2598	184.6	1.68
						5		3
						Insufficient DNA		Fail QC

Hybridization of 96 tumor samples: We have hybridized 48 samples, while other 48 are currently being hybridized at the core facility. The data are being analyzed (real-time) to help reinforce the power calculation of our study and determination of the optimal ratio of recurrent versus non recurrent tumors to be used for the training stage of the study. This is important to avoid using an excessive number of samples that can be other ways used for other studies. Overall during the next 3 months we expect to finish analysis of CNV on 300 samples, so that integration analysis with RNAseq can initiate.

Plans for the next reporting period to accomplish the goals: Finish analysis of DNA CNV on 300 samples and integrated analysis of the copy number variation result and the RNAseq results obtained from a paralleled DOD study of the PI (W81XWH-12-1-0521).

Results disseminated to communities of interest: We have created a news letter that is being distributed every 2 months to communities of interest (attached). This news letter updates the communities on the status of the project and keeps them engaged. It may be used to ask for more material. Please find attached the first version of the letter that was submitted when this project started.

Actual or anticipated problems or delays and actions or plans to resolve them: None

IMPACT

Impact on the development of the principal discipline(s) of the project: Creation of a well annotated biorepository of early-stage tumors allows performing correlative clinical and genomic studies on these tumors that are so poorly characterized and yet significantly affect the life of so many women.

Impact on other disciplines: Nothing to report

Impact on technology transfer: We anticipate that genomic discoveries in this project will have commercial application.

Impact on society beyond science and technology: Nothing to report

INTEGRATED GENOMIC ANALYSIS OF EARLY STAGE HIGH GRADE OVARIAN CANCER

Newsletter

Early Stages Ovarian Cancer consortium

Coordinating Center:
Massachusetts General Hospital
Principal Investigator:
Michael J. Birrer

Background

The standard of care for high-grade early stage ovarian cancers remains identical to their advanced stage counterparts. Platinum based therapy only benefits 50% of women with early-stage ovarian cancer resulting in **over treatment** and unnecessarily exposure to **toxic side effects for the remaining 50%** of women with this disease.

Objective

We have received a Federal Fund to create a biorepository of FFPE early-stage high-grade ovarian cancers to perform a multi- component genomic analysis including: **DNA copy number variation**, **RNA sequencing**.
We hypothesize that through integration of these multi-demonstrational genomic data we will be able to identify and validate very specific markers that distinguish recurrent from non-recurrent high-grade early-stage OCs.

Winter is coming, samples are being collected!

We are now obtaining samples from yours and from other institutions. Our last update just before this issue is **up to 175 samples.**
Our goal is to collect 200 recurrent and 200 non recurrent FFPE tumor tissues with 5 years medical follow up (if not relapsed cases).

What we need

- FFPE samples;
- early stage/high grade epithelial ovarian cancer (grade2 and 3 for old WHO classification);
- serous or endometrial histology (mixed cells tumors with at least one of these histology features represented are also eligible);
- from patients that had recurrences or not, with 5 years follow up (if not relapsed cases) and clinical data.

If you still don't have our eProtocol form, you can find it attached. You can send us block or RNAse free cut slides, please see the protocol.

What we are now doing

The Pilot study had just started. We were able to extract Nucleic Acids from each of sample obtaining a reasonable amount of DNA and RNA.

…continues….

What we need you to do:

Thank you for your collaboration, we are briefly moving forward on that project and any help will be really valued!

When you find samples in your Institution /group the following steps will be:

Submit and obtain an approval from your IRB the form you can find here attached:

- IRB form for submission
- IRB 2014 Approval Update

Fill the eProtocol form from page 3 for each sample you are going to send and, if it is possible, send also the original Pathologic report;
Contact us to arrange the Shipment!

If you request help for samples inclusion criteria, section protocol, IRB documentation, samples shipment, please send an email to lceppi@mgh.harvard.edu

Early Stage Ovarian Cancer Consortium, Massachusetts General Hospital.
Do not reply to this email, please send all the correspondence to: Lorenzo Ceppi at lceppi@mgh.harvard.edu